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Expression of Some Molybdoenzyme Genes under Salt Stress Conditions in Chickpea, Bean and Lentil Plants

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Abstract— *The objective of this work is to isolate mARC, XDH1, AAO3, NIA1, SO and ABA3/LOS5 genes in three legume species submitted to salt stress with molybdenum and nitrogen, the growth conditions (T1: control, T2: both molybdenum and nitrogen added, T3: sodium chloride were added, T4: molybdenum, nitrogen and sodium chloride were added), we found these gene sequences in Phaseolus vulgaris and Cicer arietinum in NCBI but not forLens culinaris, so we tried to isolate them using bean and chickpea primers by reverse transcriptase PCR. In chickpea, aldehyde oxidase and xanthine dehydrogenase genes expression is enhanced by molybdenum and nitrogen. Nitrate reductase gene expression is affected by salinity. Sulfite oxidase and xanthine dehydrogenase are activated under salt stress in bean, which suggests that SO and XDH protein have a role in bean adaptation to salt stress. mARC gene expression is stimulated by presence of molybdenum and nitrogen, mARC2 bean protein and mARC chickpea protein may have a role in salt stress adaptation mechanism.*

Keywords—molybdoenzymes, legumes, salt stress, reverse transcriptase PCR.

Abbreviation—*M: marker, mARC: mitochondrial amidoximereducing component, XDH1:xanthine dehydrogenase gene, AO: aldehyde oxidase gene, NIA1: nitrate reductasegene, SO:Sulfite oxidasegene, ABA3/ LOS5: Molybdenum cofactor sulfurasegene.*

I. INTRODUCTION

Mineral nutrient deficiencies and salinity constitute major limitations for crop plant growth on agricultural soils around the world (Maathuis *et al*., 2003; Tester and Davenport, 2003)

In the Maghreb more than 30 % of irrigation waters are loaded in salt, and lead over time to an accumulation of toxins both in rhizosphere and in different parts of plant. These toxins generate damage to cellular ultrastructures by contributing to a reduction of growth and yields of sensitive varieties (Rahmoune*et al.*,2008).

In Algeria, 4/5 of lands is desert and the 1/5 left is an arid and semi-arid region (Abdelaguerfi and Ramdane, 2003) and 3,2 million hectares of soil are affected by the process of salinization. (Benmahioul et *al.*, 2009).

Salt tolerance is a multigenic trait, which involves a complex of responses at metabolic, cellular, molecular, physiological and whole-plant levels, (Das et al., 2015)

We concentrate our work on food legumes because of their nutritional value is gaining considerable interest in developed countries because of the demand for healthy foods. Grains are rich in proteins, carbohydrates, and dietary fibers and are a rich source of other nutritional components (Tharanathan and Mahadevamma, 2003 and Gupta et al., 2006). Their consumption and production extends worldwide. Common seeds, such as bean (*Phaseolus vulgaris*), lentil (*Lens culinaris*), pea (*Pisumsativum*), chickpea (*Cicer arietinum*), and faba bean (*Viciafaba*,) are the most widely consumed legumes throughout the Mediterranean area and are the most cultivated legumes in Algeria. (Bouchnak and Benlamri, 2013).

In eukaryotes, the mostprominent Mo-enzymes are sulfite oxidase, whichcatalyzes the final step in the degradation of sulfur-containingaminoacids and isinvolvedin detoxifyingexcess sulfite, xanthine dehydrogenase, whichisinvolved in purine catabolism and reactiveoxygen production, aldehydeoxidase, whichoxidizes a variety of aldehydes and is essential for the biosynthesis of the phytohormone abscisicacid, and in autotrophicorganismsalso nitrate reductase, whichcatalyzes the keystep in inorganicnitrogenssimilation.(Mendel and Bittner 2006).The mitochondrial amidoxime reducing component (mARC) has been identified in mitochondria of mammals and catalyzes the reduction of *N*-hydroxylated substances (Havemeyer et al.,2011).

In this work we focus on changes that occur on XDH, AO, NR, SO, ABA3 and mARC genes expression in bean, chickpea and lentil under salt stress and presence of both molybdenum and nitrogen, using a reverse

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transcriptase PCR and actin as housekeeping gene to try to observe any difference between the species and the treatments.

II. EXPERIMENTS

2.1. Growth conditions: seeds of *Phaseolus vulgaris* L., *Cicer arietinum* L. and *Lens culinaris* M. were grown in pots with compost, the 2 weeks seedlings are submitted to 4 different treatments during 3 weeks of: 6g/l of sodium chloride, 0,2ppm of molybdenum as ammonium molybdate and 0,2g/l of nitrogen as nitrate potassium that were added to the irrigation water. The treatments correspond to: **T1**: control, **T2:** both molybdenum (Mo) and nitrogen (N) were added to the water irrigation, **T3:** only sodium chloride (NaCl) were added to the water irrigation, **T4:**: both molybdenum, nitrogen and sodium chloride were added to the water irrigation, the photoperiod night/day is 14 h/10h, the mean temperature in 23°C.

2.2. RNA isolation: we used a nucleospin RNA plant kit of Macherey-Nagel; 100mg of shoot plant material of *Phaseolus vulgaris*, *Cicer arietinum* and *Lens culinaris* were used.

2.3. Reverse transcriptase reaction: using PromegaAMV Reverse Transcriptase kit; take the volume that correspond to 60ng/µl from each species and treatment, denaturation at 70°C for 5min then we add 10ul of: 4ul de 5x AMV buffer, dNTP (10mM), RNases inhibitors (4U/ml; Promega), poly anchor primer (100pmol/ml), AMV reverse transcriptase (10U/ml).

2.4. Reverse transcriptase PCR: using GoTaq® PCR Core Systems kit from promega; In a sterile 100µl tubes put: 14ul sterile H2O, 5ul kit 5x reaction buffer, dNTP (10mM), 0,25ul forward primer (100µM), 0,25ul reverse primer(100µM), 3ul RT-reactions from: DNAcof *Phaseolus vulgaris*, *Cicer arietinum* and *Lens culinaris*under 4 different treatments: T1, T2, T3 and T4 and 0,5ul taq polymerase (2,5U).

We used Actin as housekeeping gene, 60°C as annealing temperature and 33cycles.

We looked for mARC, actin, XDH1, AAO3, NIA1, SO and ABA3/ LOS5 genes sequences on NCBI (NCBI,http://www.ncbi.nlm.nih.gov), using the sequence of their polypeptides in*Arabidopsis thaliana*

We could find these sequences on NCBI only for *Phaseolus vulgaris*, *Cicer arietinum*, but not for *Lens culinaris*, so we could design the primers for each gene for bean and chickpea (table 1 and 2) and used them also for lentil.The PCR program is: 5min: 95°C,{1min: 94°C, 1min: 60°C annealing temperature, 1min: 72°C} 33cycles, 7min: 72°C and hold at 4°C.

The PCRproduct were separated on a 1,5% agarose gel using a DNA size marker, the bands of interest (that correspond to 500pb as the size of the genes used) from *Lens culinaris* were excised, purified and cloned in *E.coli* DHα5 and sequenced . The gel photos were produced by intasscience imaging GDS.

2.5. Purification: using PCR clean up Gel extraction kit, we extract and purify our interesting DNA from the agarose gel. Ligation: using Thermo Scientific CloneJET PCR Cloning Kit; Reaction buffer : 10µl, DNA insert : 8,5µl, the PJET1.2 plasmid: 0,5µl, DNA ligase : 1µl, meanwhile check the DNA insert on 1,5% agarose gel. Incubation at 22°C for 30min.

Transformation: we used aliquots of 100µl of competent *E.coli* DH5α bacteria, Plasmid isolation and DNA insert purification from *E. coli*: isolation of High copy plasmid DNA from *E. coli* (kit nucleospin plasmid DNA purification), and check the insert DNA on 1,5% agarose gel

2.6. Digestion :using Thermo Scientific FastDigestBglII kit; H2O dd : 3,5ul, FD digestion buffer: 1ul, BGLII enzyme: 0,5ul, eluted DNA: 5ul, incubation at 37°C for 30min and check the eluted DNA on 1,5% agarose gel, and send the DNA of interest to be sequenced using Eurofins Genomics DNA sequencing service.

2.7. Statistical analysis:

The data obtained were assessed by one way anova, tables (3) and (4) were obtained using xlstat at the confidence level of 95%, and using imageJ to estimate band intensity.

Table.1: Primers sequences used for Phaseolus vulgaris and Lens culinaris

Protein	Gene	Nucleotides accession number	Primers
Actin	**Actin**	KF569629	Forward:5'-AGGTTATTCCTTCACTACCACCGC-3' Reverse:5'-AGCCTCATCATACTCAGACTTTGC-3'
mARC	**mARC1**	XM_007138795	Forward:5'-CACTTGTTGAACACTATCAACCTACCAAC-3' Reverse: 5'-CCTTGTTTTCATAAGAGTCTCAGTTGGC-3'
mARC	**mARC2**	XM_007138794	Forward: 5'-CTTGAAAACTGGGAACCAACTCAAGAC-3' Reverse: 5'-CATGAGAATTTCATTTGTTTCTGGC-3'

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AO	**AAO3**	XM_007141542	Forward:5'-TGC CTT CAA TAT GGA GTA AGT TGG C-3' Reverse:5'-TAA GTT CAC AGC TCG CAG GTT TGC C-3'
XDH	**XDH1**	XM_007150309	Forward:5'-CGA ATA GAT CTT TCT GCC CAT GG-3' Reverse:5'-TCC GAA TTC TCT CTG GAG TTG C-3'
NR	**NIA1**	XM_007141046	Forward:5'-CCT ACA CTC CAA CAA GTA GCG-3' Reverse:5'-AGA ATC GCT TGA AGC TTC TGG -3'
SO	**SO**	XM_007151463	Forward:5'-GTG TCA TTG GAG CTC GAT CTG-3' Reverse:5'-GGT TTG AGT GAC TTG CTT GGA C-3'
ABA3	**ABA3 /LOS5**	XM_007133810	Forward:5'-AGC AAT GGT CTG ACA CAT GAT CGC-3' Reverse:5'-TCG CTA TTG CTC TTC CAG ATA TGC C-3'

Table.2: Primers sequences used for Cicer arietinum and Lens culinaris

Protein	**Gene**	Accessions number	Primer
Actin	**Actin**	XM_004497837	Forward:5'-AACTGGTATTGTTCTGGATTCCGG-3' Reverse:5'-TTCATGCTACTTGGTGCCAATGC-3'
mARC	**mARC**	XM_004487841	Forward:5'-GTAGACCCTGATTATGTTGAGGAACAG-3' Reverse:5'-TCAAGCAGCTGCTTCTGCTGCAGAAG-3'
AO	**AAO3**	XM_004491092	Forward:5'-TAC ATA CCT AGC AAT AAC TCG ATG-3' Reverse:5'-CAG GTC CTT CTT CGT TGC TCC-3'
XDH	**XDH1**	XM_004486904	Forward:5'-TGC TAT GCA GAG CGA ATA GAC C-3' Reverse:5'-CAA CTC TTG CAG CAC TGA TGG C-3'
NR	**NIA1**	XM_004513774	Forward:5'-CTT ATA CTC CAA CGA GTA GTG TCG-3' Reverse:5'-CAA AGC CAA AGT ATC CTG ACT TGC-3'
SO	**SO**	XM_004489554	Forward:5'-GGT CTG TTA AAT GGC TGG AAG C-3' Reverse:5'-CTT GAA CTT GGA CTC GAT GCC-3'
ABA3	**ABA3/ LOS5**	XM_004506406	Forward:5'-AGT TAC AGC AAC GAG ACC AAT GC-3' Reverse:5'-AGC CAA GAA TCA CCT TGT TGC TGC-3'

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III. RESULTS

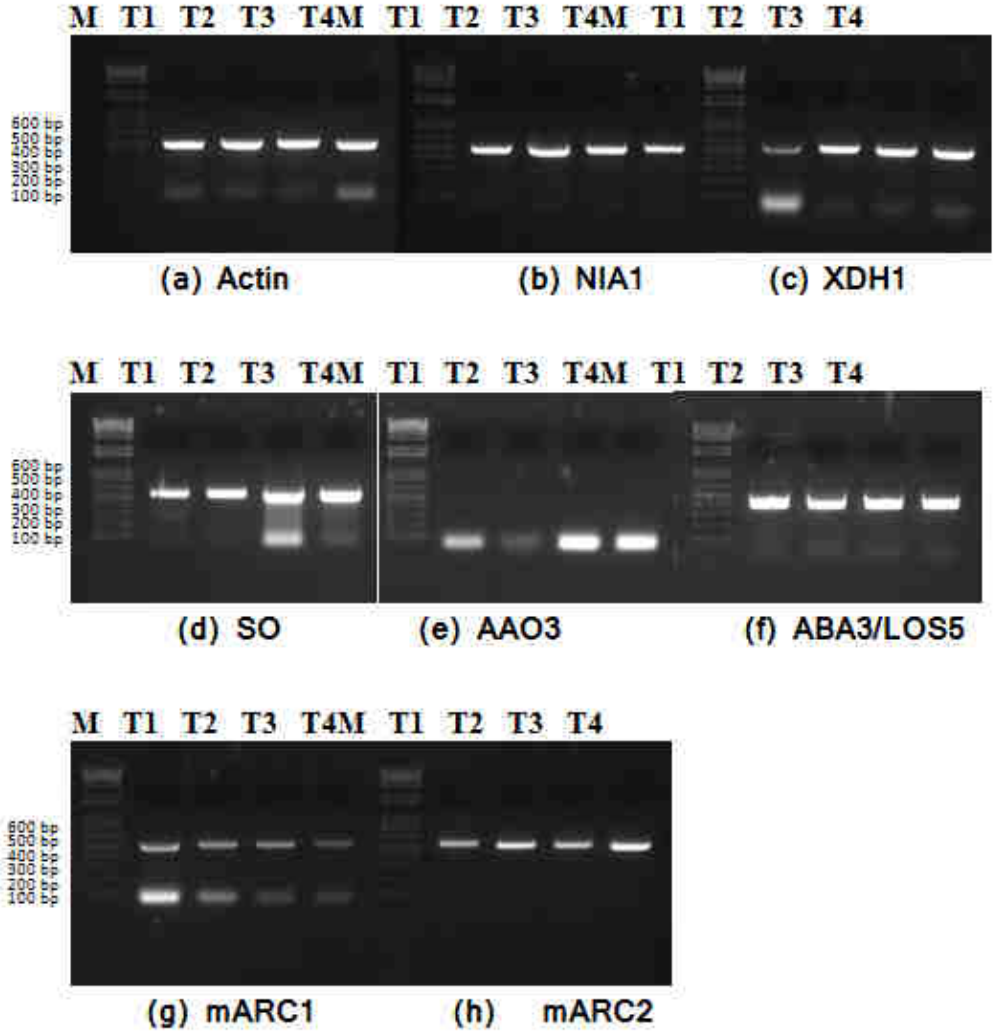

Fig.1: Gene expression on agarose gel electrophoresis of reverse transcriptase (RT) PCR of actin (a), NIA1 (b), XDH1(c), SO (d), AAO3 (e), ABA3/LOS5 (f), mARC1 (g) and mARC2 (h) in bean plants (Phaseolus vulgaris) under different treatments (M: marker, T1: control, T2: both Mo and N added, T3: only NaCl added, T4: Mo, N and NaCl added)

Table.3: Intensity of gene expression of NIA1, XDH1, SO, AAO3, ABA3/LOS5, mARC1 and mARC2 in Phaseolus vulgaris plants under different treatments using image

	NIA1	XDH1	SO	ABA3/LOS5	mARC1	mARC2
T1	0,97 b ± 0,010	0,39 d ± 0,013	0,67 d ± 0,066	**1,13 a**± 0,008	**1,031a** ± 0,008	0,678 c ± 0,023
T2	**1,09 a**± 0,013	0,88 c ± 0,016	0,72 c ± 0,012	0,93b ± 0,008	0,739 b ± 0,006	0,820 b ± 0,012
T3	0,93 c ± 0,026	0,96 b ± 0,045	1,04 b ± 0,035	0,87 c ± 0,008	0,569 c ± 0,010	0,651 d ± 0,005
T4	0,80 d ± 0,018	**1,06 a**± 0,033	**1,09 a**± 0,015	0,86 c ± 0,008	0,350 d ± 0,013	**1,021a** ± 0,018

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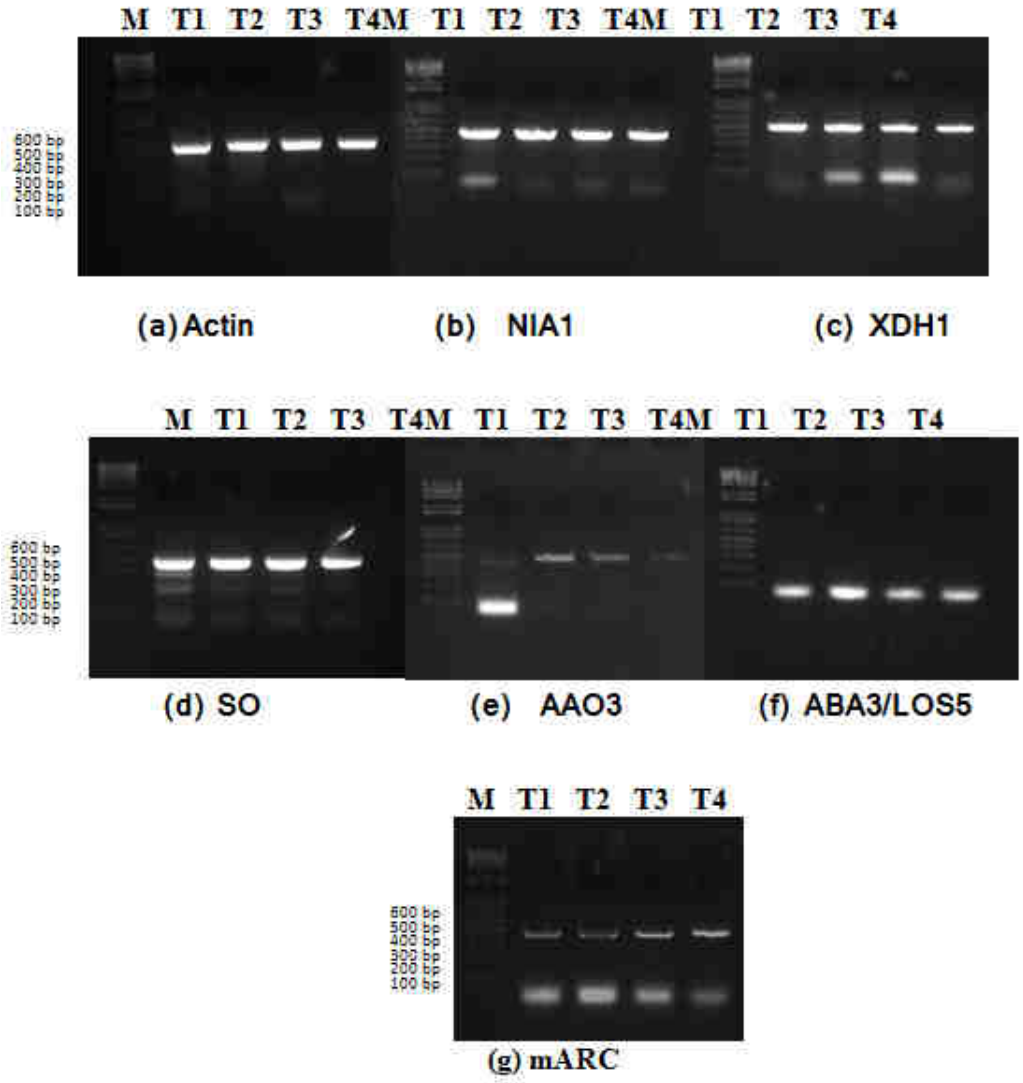

Fig.2:Gene expression on agarose gel electrophoresis of reverse transcriptase (RT) PCR of actin (a), NIA1 (b), XDH1(c), SO (d), AAO3 (e), ABA3/LOS5 (f) and mARC (g) in chickpea plants(Cicer arietinum) under different treatments (M: marker, T1: control, T2: both Mo and N added, T3: only NaCl added, T4: Mo, N and NaCl added)

Table.4: Intensity of gene expression of NIA1, XDH1, SO, AAO3, ABA3/LOS5 and mARC in Cicer arietinum plants under different treatments using imageJ

	NIA1	XDH1	SO	AAO3	mARC
T1	0,95b ± 0,006	0,85 b± 0,028	**1,028a**± 0,022	0,351 d ± 0,023	0,561 c ± 0,018
T2	**0,98a**± 0,016	**0,93a**± 0,022	0,865 b± 0,018	**0,900a**± 0,054	0,471 d ± 0,025
T3	0,918c ± 0,033	0,778 c± 0,034	0,858b ± 0,018	0,661 b ± 0,014	0,759 b ± 0,015
T4	0,838d ± 0,024	0,762 c± 0,009	0,898 b± 0,037	0,462c ± 0,052	**1,088 a** ± 0,023

We looked for all this studied genes sequences for *Lens culinaris* in ncbi and we didn't find any, so we tried to isolate them using the same primers we used for bean and chickpea.

Figure 3 and 4 show an expression of SO, XDH1 and mARC. These cDNA were purified and cloned in DH5α, and sequenced.

http://dx.doi.org/10.22161/ijeab/1.4.20 *ISSN: 2456-1878*

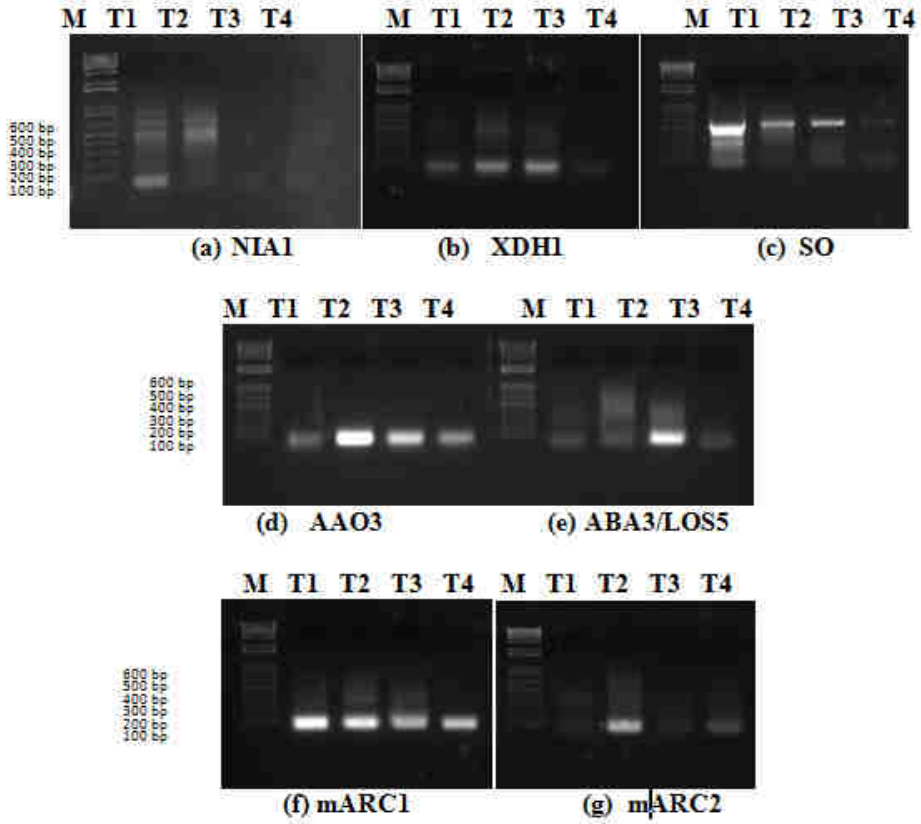

Fig.3: Gene expression on agarose gel electrophoresis of reverse transcriptase (RT) PCR of NIA1 (a), XDH1 (b), SO (c), AAO3 (d), ABA3/LOS5 (e), mARC1 (f) and mARC2 (g) in lentil plants(Lens culinaris) using bean primers

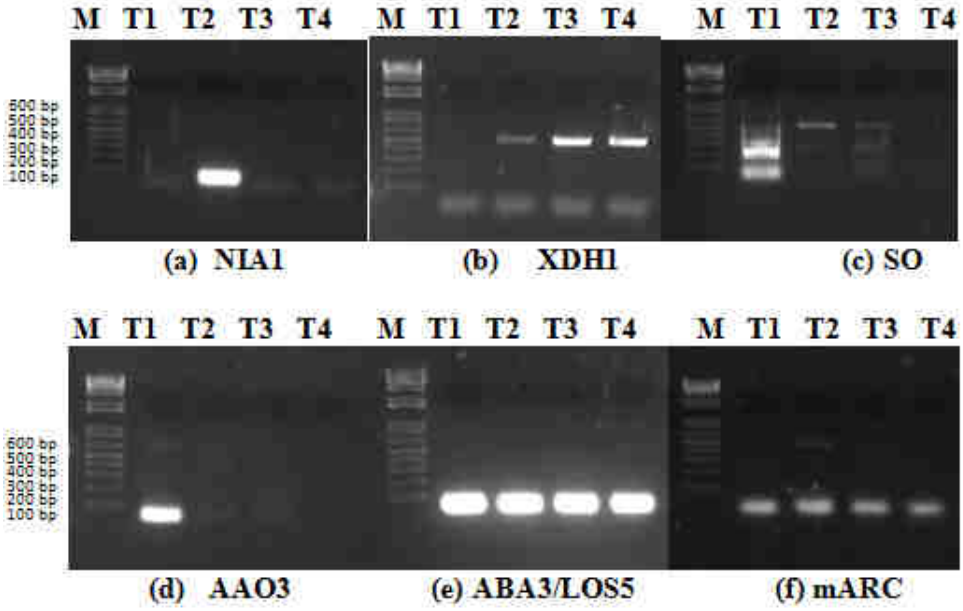

Fig.4: Gene expression on agarose gel electrophoresis of reverse transcriptase (RT) PCR of NIA1 (a), XDH1 (b), SO (c), AAO3 (d), ABA3/LOS5 (e), mARC1 (f) and mARC2 (g) in lentil plants(Lens culinaris) using chickpea primers.

IV. DISCUSSION

Molybdoenzymes in plants are key enzymes in nitrate assimilation, purinemetabolism, hormonebiosynthesis, and mostprobably in sulphitedetoxification. They are considered to be involved instress acclimation processes and, therefore, elucidation of the mechanisms of their response to environmental stress conditions is of agricultural importance for the improvement of plant stress tolerance.(Zdunek-ZastockaandLips, 2003)

Twodifferentmolybdenum enzyme families are known ineukaryotes (Hille et *al*., 2011): (i) the sulfite oxidasefamily, to whichalsonitrate reductaseand the

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mitochondrial amidoxime-reducing component belong,and the (ii) xanthine oxidasefamily, to which also aldehydeoxidase belongs. In bacteria, a third class of molybdenumenzymes isknown in whichtwo MPT equivalentscoordinateone molybdenumatom (Magalon et *al*., 2011). It isassumedthat the rare eukaryoticmolybdenum enzymes pyridoxal oxidase (Warner and Finnerty, 1981) and nicotinatehydroxylase (Lewis et *al*., 1978)representspecificisoforms of aldehydeoxidase. In contrast to the sulfite oxidasefamily, the membersof the xanthine oxidasefamilyrequire a final step of maturationprior to or after insertion of molybdenum cofactor (Moco). In addition to the dithiolenesulfurs of the pterinmoiety and two oxo groups, the molybdenumatom of Moco needs the addition of a terminal inorganicsulfur to provide enzyme activity to these enzymes (Wahl et *al*., 1984). This final step is catalyzed by the molybdenum cofactor sulfurase protein (ABA3)

Osmotic stress resultingfromeitherhighsalinity or water deficitinduces the expression of numerous stress-responsivegenes in plants (Xiong et *al*., 2002).

Figure 1, 2 and 3 show the expression of molybdoenzyme genes under salt stress and in presence of molybdenum and nitrogen in irrigation water, this expression differs between treatments and species.

Aldehydeoxidase (AO) has derivedfrom XDH by gene duplicationand neo-functionalizationand thereforesharescatalytical andstructural similaritieswithXDH. In contrast to XDH however, AO proteinspreferablyoxidizealdehydes to the respective carboxylicacid. Moreover, molecular. (Bittner, 2014)

Aldehyde oxidase (AO; EC 1.2.3.1) and xanthine dehydrogenase (XDH; EC 1.2.1.37) are known to take part in processes connected with the adaptation of living organisms to stress conditions. So, for example, AOcatalyzes the last stages in biosynthesis of two phytohormones; oxidation of abscisic aldehyde up to abscisic acid and oxidation of indole-3-acetaldehyde up to indole-3-acetic acid (Walker-Simmons et *al*, 1989, and Koshiba et *al*., 1996). XDH takes part in a purine metabolism and also in biosynthesis of ureides in higher plants; ureides like urea, as “scavengers”, could remove oxygen radicals, which are formed under stress conditions (Sagi et *al*., 1998)

Aldehyde oxidase gene (AAO3) doesn’t show any expression in bean plants but in chickpea we can observe an intense expression when molybdenum and nitrogen are added to plant irrigation water, (Tab.4).The most important isoformis AAO3,whichcatalyzes the oxidation of abscisicaldehyde to abscisicacid(ABA) in the last cytosolicstep of ABA synthesis. Due to the function of ABA in many aspects of plant growth and development, andin adaptation to a variety of abiotic stresses, AAO3-deficient plantswithreduced ABA levels are characterized by a high transpirationrate, reduced stress tolerance, and impairedseeddormancy (Seoand Koshiba, 2011 in Bittner (2014)).

Xanthine dehydrogenase (XDH1) is well expressed in bean plants under salt stress and with Mo and N added, (Table 3) and has the lowest expression in control plants, but in chickpea the highest expression of this gene is observed in plants receiving Mo and N in their water irrigation without any stress condition (Table 4). XDH activitywasenhanced by salinity andammonium in maize nodal roots (Barabás*et al.* 2000 in Zdunek-ZastockaandLips, (2003)). Increase of XDH undersimilar conditionswaspreviouslyreported in ryegrass, whereitcorrelatedwith a higher content of ureides in plant tissues (Sagi*et al.* 1998).

Statistics in table 3 show adding Mo and N in irrigation water for bean plants leads to strong expression of nitrate reductase gene (NIA1) that decrease when plants are exposed to salt stress, nitrate reductase is an enzyme affected by salinity, the same is observed for chickpea plants (Table 4).

Sulfite oxidase gene (SO) is highly expressed under salt stress with Mo and N in bean plants, this expression decrease with reduction of salinity as the lowest value in table 3 is noted for control plants. In chickpea plants we observe the important expression of SO gene for control plants, and for the other treatments there is no difference between the band intensity values (Table 4).

Sulfite oxidase catalyzes the oxidationfrom sulfite to sulfate, the final step in the degradation ofsulfur-containingaminoacids. (Mendel and Bittner, 2006), SO isaperoxisomal enzyme(Nowak et al. 2004), whichexclusivelyconsists of a Moco-binding domain required for oxidizingsulfite to sulfate(Bitnner, 2014). As sulfite is a strongnucleophilethatcanreactwith a widevariety ofcellular components, itwasassumedthat SO isrequired for removingexcess sulfitefrom the cell. (Bittner and Mendel, 2010)

The Molybdenumco factor sulfurase (ABA3)is a homodimerictwo-domainprotein(Bittner et *al*., 2001), that activate AO and XDH enzymes (Bittner, 2014)

In bean, ABA3/LOS5 gene expression is affected by salinity, as we note on Table 3, control plants have the most important value of band intensity, this expression in affected by salinity, but not enhanced by Mo or N added. In chickpea we couldn’t observe any expression of the gene.

Moco sulfurase catalyzes the generation of the sulfurylated form of Moco, a cofactor required by aldehyde oxidase that functions in the last step of ABA

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biosynthesis in plants. The LOS5/ABA3 gene is expressed ubiquitously in different plant parts, and the expression level increases in response to drought, salt, or ABA treatment.(Xion et *al*., 2001)

Like mammals, plant genomes encode two mARC isoforms, which have not yet been investigated in detail. The physiological role of mARC proteins is therefore still enigmatic, even though previous studies in *Chlamydomonas*and on recombinant human proteins suggest a function in the detoxification of *N*-hydroxylated base analogs (Chamizo-Ampudiaetal.,2011; Krompholz et al.,2012 in Bittner, 2014) and/or in the regulation of L-arginine-dependent NO synthesis (Kotthausetal.,2011 in Bittner, 2014). the difference between the two mARC isoforms gene expression in bean plants with the expression of the housekeeping gene actin, obviously the two isoform express differently, we used ImageJ to better appreciate the intensity (table.3)

Research on NCBI leads us to two mARC isoforms; mARC1 and mARC2 in bean species, early work on mARC indicates that depending on species, usually one isoform is predominantly expressed (Plitzko et al., 2013), as we can see (figure. 2) the two isoform express differently, the highest value of mARC1 intensity is 1,031represented by the control, that means that adding molybdenum or nitrogen doesn't enhance mARC1 gene expression, and salt stress may lead to a decrease in its protein expression, the contrary happens in mARC2; so we can observe that the most important value is 1,021 (table3) registered for bean plant that are submitted to a salt stress adding molybdenum and nitrogen, comparing to the lowest value which represent control plants.

For chickpea, research on NCBI lead us to one form, mARC, which was isolated using same methods, mARC gene expression is almost the same than mARC2 in bean which shows a higher band intensity in T4 (figure. 3) with the most important value of 1,088 (table. 4), it seems that mARC protein in chickpea and mARC2 protein in bean may have a role in salt stress adaptation mechanism.

As we can see on figure 3 and 4 in lentil we could isolate just SO, XDH1 and mARC genes and we succeed in sequencing only 2 genes; XDH1 and mARC

Sequencing results:

mARC gene

GTGGCTCGAGTTTTTCAACAAGATTAGACCCTGA
TTATGTTGAGGAACAGCAGATGACCTTGTTCAGT
GATGGTTATCCATTCTTACTTGTATCTCAGGATTC
ACTGGATGCACTAAACAAGCTTCTAGACGAATCC
ATATCTATGAATCGTTTCAGACCCAATATCCTTG
TTGAAGGTTGTGAAGCATATTCTGAAGACTTGTG
GAGAGATATCAAGATAAGCAGGTTTTCATTTCAG
GGTGTCAAGCTGTGTGCCCGTTGTAAGGTGCCAA
CAATCAATCAAGAGACAGCAATACATGGAACTG
AACCATATGAAACTCTCACGAAAGTTCGGTCTGG
CGAAGTCTTGAGACCAAATAAAAAAAACAAAAA
AAAGATCTACTTTGGTCAGAATGTAGTGTGGAAC
TGGAAGGATTCTTCTGCTAAAGGGGATGGAAAC
GTGCTTAAACTGGGAGATCCTGTTTATGTTATCA
AAACATATTCTTCTGCAGCAGAAGCAGCTGCTTG
AATCTTTCTAGAAGATCTCCTACAATATTCTCAG
CTGCCATGGAAAATCGATGTTCTTCTTTTATTCTC
TCAAGATTTTCAGGCTGTATATTAAAACTTATAT
TAAGAACTATGCTAACCACCTCATCAGGAACCGT
TGTAGGTGGCGTGGGTTTTCTTGGCAATCGACTC
TCATGAAAACTACGAGCTAAATATTCAATATGTT
CCTCTTGACCAACTTTATTCTGCATTTTTTTTGAA
CGAGGTTTAGAGCAAGCTTCAGGAAACTGAGAC
AGGAATTTTATTAAAAATTTAAATTTTGAAGAAA
GTTCAGGGTTAATAGCATCCATTTTTTGCTTTGCA
AGTTCCTCAGCATTCTTAACAAAAGACGTCT

This sequence were submitted to:
http://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch&PROG_DEF=blastn&BLAST_PROG_DEF=megaBlast&BLAST_SPEC=blast2seqand
and were compared to the 489 pb sequence of chickpea XM_004487841 (Cicer arietinum mitochondrial amidoxime reducing component 2-like LOC101506896, mRNA):

GTAGACCCTGATTATGTTGAGGAACAGCACCAG
ACCTTGTTCACTGATGGTTATCCGTTCTTACTCAT
ATCTCAAGAATCGTTGGATGCAGTAAACAAGCTT
CTAGAGGAACCGATACCTATGAATCGTTTCAGAC
CCAATATCCTTGTTGAAGGTTGTGAACCATTTTCT
GAAGACATATGGAGAGATATCAAGATAAGCAGG
TTTTCATTTCAGGGTGTCAAGCTGTGTTCCCGTTG
TAAGGTACCAACAATAAACCAAGAGACAGCAAT
ACCAGATGGATCTGAACCAACTGAAACTCTCATG
AAAATTAGGTCTGGCAAAGTCATAAGACCAAAT
GATAAAAACAAAAACAAGGTCTACTTTGGTCAG
AATATAGTCTGGAATTGGAGGGATTCTTCTGCTA
AAGGGGATGGAAAAGTCCTTAAAGTTGGAGATC
CAGTTTATGTTACCAAAAAGTTATCTTCTGCAGC
AGAAGCAGCTGCTTGA

And the result of the alignment is:

Alignmentstatistics for match #1

Score	Expect	Identities	Gaps	Strand
599 bits(324)	1e-175	434/488(89%)	3/488(0%)	Plus/Plus

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Query 25
TAGACCCTGATTATGTTGAGGAACAGCAGATGAC
CTTGTTCAGTGATGGTTATCCATTCT 84
Sbjct 2
TAGACCCTGATTATGTTGAGGAACAGCACCAGAC
CTTGTTCACTGATGGTTATCCGTTCT 61
Query 85
TACTTGTATCTCAGGATTCACTGGATGCACTAAA
CAAGCTTCTAGACGAATCCATATCTA 144
Sbjct 62
TACTCATATCTCAAGAATCGTTGGATGCAGTAAA
CAAGCTTCTAGAGGAACCGATACCTA 121
Query 145
TGAATCGTTTCAGACCCAATATCCTTGTTGAAGG
TTGTGAAGCATATTCTGAAGACTTGT 204
Sbjct 122
TGAATCGTTTCAGACCCAATATCCTTGTTGAAGG
TTGTGAACCATTTTCTGAAGACATAT 181

Query 205
GGAGAGATATCAAGATAAGCAGGTTTTCATTTCA
GGGTGTCAAGCTGTGTGCCCGTTGTA 264
Sbjct 182
GGAGAGATATCAAGATAAGCAGGTTTTCATTTCA
GGGTGTCAAGCTGTGTTCCCGTTGTA 241

Query 265
AGGTGCCAACAATCAATCAAGAGACAGCAATAC-
--ATGGAACTGAACCATATGAAACTC 321
Sbjct 242
AGGTACCAACAATAAACCAAGAGACAGCAATAC
CAGATGGATCTGAACCAACTGAAACTC 301

Query 322
TCACGAAAGTTCGGTCTGGCGAAGTCTTGAGACC
AAATaaaaaaaacaaaaaaaGATCT 381
Sbjct 302
TCATGAAAATTAGGTCTGGCAAAGTCATAAGACC
AAATGATAAAAACAAAAACAAGGTCT 361
Query 382
ACTTTGGTCAGAATGTAGTGTGGAACTGGAAGG
ATTCTTCTGCTAAAGGGGATGGAAACG 441
Sbjct 362
ACTTTGGTCAGAATATAGTCTGGAATTGGAGGGA
TTCTTCTGCTAAAGGGGATGGAAAAG 421

Query 442
TGCTTAAACTGGGAGATCCTGTTTATGTTATCAA
AACATATTCTTCTGCAGCAGAAGCAG 501
Sbjct 422
TCCTTAAAGTTGGAGATCCAGTTTATGTTACCAA
AAAGTTATCTTCTGCAGCAGAAGCAG 481

Query 502 CTGCTTGA 509
Sbjct 482 CTGCTTGA 489

XDH1 gene

GTGGCTCGAGTTTTTCAGCAAGATTGCTATGCAG
AGCGAATAGACCTTTCTGCCCATGGA
TTTTATATTACACCTGATATTGGCTTTGATTGGAT
CACGGGTAAAGGAAAACCTTTTAGG
TATTTCACTTACGGGGCTGCATTTGCCGAGGTTG
AAATTGACACCTTGACTGGAGATTTT
CACACCAGGGTGGCAGACATAATTTTGGATCTCG
GTTATTCTCTGAACCCAGCAATAGAT
GTTGGGCAGATCGAAGGAGCTTTTATTCAAGGTT
TGGGCTGGGTTGCTTTAGAAGAACTT
AAATGGGGAGATGCAGCTCATAAATGGATCCCC
TCTGGGTGGCTTAACACTTGTGGACCC
GGAGCTTATAAAATTCCTTCTATAAATGACGTTC
CCTTGAAATTTGATGTCTCACTTCTG
AAGGGCCATCCAAATGTAAAGGCAATCCATTCGT
CTAAAGCAGTTGGCGAGCCTCCGTTT
TTCTTAGCATCAGCTGTATTCTTTGCCATAAAGG
ATGCCATCAGTGCTGCAAGAGTTGAT
CTTTCTAGAAGATCTCCTACAATATTCTCAGCTG
CCATGGAAAATCGATGTTCTTCTTTT
ATTCTCTCAAGATTTTCAGGCTGTATATTAAAAC
TTATATTAAGAACTATGCTAACCACC
TCATCAGGAACCGTTGTAGGTGGCGGGGGGTTTT
CTTGGCAATCGACTCTCATGAAAACT
ACGAGCTAAATATTCAATATGTTCCTCTTGACCA
ACTTTATTCTGCATTTTTTTTGAACG
AGGTTTAGAGCAAGCTTCAGGAAACTGAGACAG
GAATTTTATTAAAAATTTAAATTTTGA
AGAAAGTTCAGGGTTAATAGCATCCATTTTTTGC
TTTGCAAGTTCCTCAGCATTCTTAAC
AAA

This sequence were submitted to:
http://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=MegaBlast&PROGRAM=blastn&BLAST_PROGRAMS=megaBlast&PAGE_TYPE=BlastSearch&BLAST_SPEC=blast2seq&DATABASE=n/a&QUERY=&SUBJECTS=

http://dx.doi.org/10.22161/ijeab/1.4.20 *ISSN: 2456-1878*

and compared to this 514pb sequence of chickpea XDH1 XM_004486904 (Cicer arietinum xanthine dehydrogenase 1-like (LOC101495267), mRNA)

TGCTATGCAGAGCGAATAGACCTTTCTGCCCATG
GTTTTTTTATTACACCTGATATTGGTTTTGA
TTGGACCACGGGTAAAGGAAACCCTTTTAGGTAT
TTCACATATGGGGCTGCATTTGCTGA
GGTTGAAATTGACACCTTGACTGGAGATTTTCAC
ACTAGGGTGGCAAATATATTTTTGGA
TCTCGGTTATTCTCTGAATCCAGCGATAGATGTT
GGGCAGATCGAAGGAGCTTTTATTCA
AGGTTTGGGTTGGGCTGCTTTAGAAGAACTTAAA
TGGGGAGATGGAGCACATAAATGGAT
CCCCTCTGGATGGCTTAACACTTGTGGACCTGGA
GCTTATAAAATTCCTTCTATAAATGA
TGTTCCCTTGAAATTTAATGTCTCACTTCTTAAGG
GCCATCCAAATGTAAAGGCAATCCA
TTCATCGAAAGCAGTTGGTGAGCCTCCATTTTTC
CTAGCATCAGCTGTATTCTTCGCCAT
CAAGGATGCCATCAGTGCTGCAAGAGTTG
And the result of the alignment is:

Alignmentstatistics for match #1

Score	Expect	Identities	Gaps	Strand
784 bits(424)	0.0	484/514(94%)	0/514(0%)	Plus/Plus

```
Query  25   TGCTATGCAGAGCGAATAGACCTTTCTGCCCATG
GATTTTATATTACACCTGATATTGGC  84
            ||||||||||||||||||||||||||||||||| |||| ||||||||||||||||
Sbjct  1    TGCTATGCAGAGCGAATAGACCTTTCTGCCCATG
GTTTTTTTATTACACCTGATATTGGT  60

Query  85   TTTGATTGGATCACGGGTAAAGGAAAACCTTTTA
GGTATTTCACTTACGGGGCTGCATTT  144
            |||||||||| |||||||||||||| |||||||||||||||| || ||||||||||
Sbjct  61   TTTGATTGGACCACGGGTAAAGGAAACCCTTTTA
GGTATTTCACATATGGGGCTGCATTT  120

Query  145  GCCGAGGTTGAAATTGACACCTTGACTGGAGATT
TTCACACCAGGGTGGCAGACATAATT  204
            || |||||||||||||||||||||||||||||||||||| |||||||| | ||| ||
Sbjct  121  GCTGAGGTTGAAATTGACACCTTGACTGGAGATT
TTCACACTAGGGTGGCAAATATATTT  180

Query  205  TTGGATCTCGGTTATTCTCTGAACCCAGCAATAG
ATGTTGGGCAGATCGAAGGAGCTTTT  264
            |||||||||||||||||||| ||||| ||||||||||||||||||||||||||
Sbjct  181  TTGGATCTCGGTTATTCTCTGAATCCAGCGATAG
ATGTTGGGCAGATCGAAGGAGCTTTT  240

Query  265  ATTCAAGGTTTGGGCTGGGTTGCTTTAGAAGAAC
TTAAATGGGGAGATGCAGCTCATAAA  324
            |||||||||||||| |||| |||||||||||||||||||||||||| ||| ||||||
Sbjct  241  ATTCAAGGTTTGGGTTGGGCTGCTTTAGAAGAAC
TTAAATGGGGAGATGGAGCACATAAA  300

Query  325  TGGATCCCCTCTGGGTGGCTTAACACTTGTGGAC
CCGGAGCTTATAAAATTCCTTCTATA  384
            |||||||||||||| |||||||||||||||||| ||||||||||||||||||||||
Sbjct  301  TGGATCCCCTCTGGATGGCTTAACACTTGTGGAC
CTGGAGCTTATAAAATTCCTTCTATA  360

Query  385  AATGACGTTCCCTTGAAATTTGATGTCTCACTTCT
GAAGGGCCATCCAAATGTAAAGGCA  444
            ||||| |||||||||||||| |||||||||||| ||||||||||||||||||||||
Sbjct  361  AATGATGTTCCCTTGAAATTTAATGTCTCACTTCT
TAAGGGCCATCCAAATGTAAAGGCA  420

Query  445  ATCCATTCGTCTAAAGCAGTTGGCGAGCCTCCGT
TTTTCTTAGCATCAGCTGTATTCTTT  504
            |||||||| || |||||||||| ||||||| |||||| ||||||||||||||||||
Sbjct  421  ATCCATTCATCGAAAGCAGTTGGTGAGCCTCCAT
TTTTCCTAGCATCAGCTGTATTCTTC  480

Query  505  GCCATAAAGGATGCCATCAGTGCTGCAAGAGTT
G  538
            ||||| ||||||||||||||||||||||||||
Sbjct  481  GCCATCAAGGATGCCATCAGTGCTGCAAGAGTTG
514
```

V. CONCLUSION

As concluding remarks, we can say that in chickpea, aldehyde oxidase and xanthine dehydrogenase gene expression is enhanced by molybdenum and nitrogen. Nitrate reductase gene expression is affected by salinity but increased by molybdenum and nitrogen in both bean and chickpea. Sulfite oxidase and xanthine dehydrogenase are activated under salt stress in bean, which suggest that SO and XDH protein have a role in bean adaptation to salt stress.

This work need to be investigated deeply, however mARC gene expression is stimulated by presence of molybdenum and nitrogen, mARC2 bean protein and mARC chickpea protein may have a role in salt stress adaptation mechanism and are stress responsive genes.

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